

Scanning electron micrograph of the small intestine of a mouse shows finger-like projections called villi underlying a layer of mucus. Villi on the inner surface of the intestine enable nutrients to be absorbed. The red arrows indicate portions of the gel-like layer of mucus that normally lines the intestine. The white scale bar at the bottom right, shown for reference, is 100 μ m long (1/10th of a millimeter, or about 1/250th of an inch). Image courtesy of Dr. Jeffrey Gordon and reprinted from Sonnenberg JL, Angenent LT, and Gordon JI. Getting a grip on things: how do communities of bacterial symbionts become established in our intestine?

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Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. These conditions include disorders of the gastrointestinal tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. Disorders of the digestive tract—such as irritable bowel syndrome and inflammatory bowel disease—exact a significant toll on many Americans each year. NIDDK-supported scientists are vigorously pursuing research to understand how widespread these diseases are across the U.S., to identify the causes of these diseases and how they progress, and to test new interventions for treatment and prevention of these costly diseases, including drugs, surgery, and behavior modification.

Several types of liver disease have serious adverse impacts on health, and some can lead to complete liver failure. Some liver diseases primarily affect children—such as biliary atresia, a progressive inflammatory liver disease—while others more commonly affect adults—such as non-alcoholic steatohepatitis (NASH). Some are caused by viral infection—such as hepatitis C—while others arise from diverse factors such as autoimmune reactions, genetic mutations, drug toxicity, and other, unknown triggers. A functioning liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. The number of livers available from deceased donors is limited, and research is of critical importance to identify and treat liver disease, preserve liver function in people with liver disease, and explore treatment options beyond cadaveric liver transplants.

The number of overweight and obese Americans has risen dramatically in the past two decades and is now at epidemic levels. Obesity is associated with numerous serious diseases, including type 2 diabetes, heart disease, and cancer. While multiple factors contribute to obesity, caloric intake clearly plays a key role in weight gain. As scientists elucidate the molecular factors that control appetite, metabolism, and energy storage, they are identifying potential targets for the development of new pharmacologic agents to promote safe, long-term weight loss. Investigators are also continuing behavioral research to help people achieve healthy lifestyles that include

increased physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the next chapter.)

Intestinal disorders include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown.

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. To address this condition, surgery may be required, including removal of the affected region of the intestine. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. Helping to catalyze the design of novel therapeutic strategies will be the continued identification of predisposing genetic variations and their interactions, as well as other factors, such as potential autoimmune and microbial influences.

The microorganisms that inhabit the gastrointestinal tract are powerful players in maintaining or tilting the balance between digestive health and disease. These microbes can affect intestinal health in some surprising ways, depending on their interactions with cells of their host. Scientists are gaining insights into the ways these microorganisms influence the development and function of the digestive tract.

Some digestive diseases can be triggered by the body's reaction to certain foods. In individuals with celiac disease, the small intestine is damaged when the immune system reacts to the protein gluten—a component of wheat, barley, and rye. This reaction interferes with the ability to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, growth failure. The only current treatment for celiac disease is maintenance of a gluten-free diet, which is difficult for many people. The greater challenge now facing patients and their healthcare providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

BOLSTERING LIVER DISEASE RESEARCH AT THE NIDDK

Action Plan for Liver Disease Research: Liver disease is an important cause of morbidity and mortality in the United States, affecting persons of all ages, but most frequently individuals in the productive years of life, between the ages of 40 and 60 years. Liver disease also disproportionately affects minority individuals and the economically disadvantaged. Medical research on liver disease is critically important and further progress in research promises to reduce the major toll of liver disease on human health and well-being. Indeed, the last 25 years of medical research in liver disease has resulted in major improvements in the survival and quality-of-life of patients with liver disease. The future should bring even more profound changes.

To address the burden of liver diseases in the United States, a subcommittee of the Digestive Diseases Interagency Coordinating Committee, led by the NIDDK, has developed an Action Plan for Liver Disease Research. The Action Plan addresses the broad range of liver disease research, and is organized around 16 topic areas. The overall goal of the Action Plan for Liver Disease Research is to advance research on liver disease with the aim of decreasing its burden in the U.S. For more information about the trans-NIH Action Plan for Liver Disease Research, see page 46.

Immune Cell Transplantation for Liver Disease of Hereditary Tyrosinemia Type I: New approaches for treating liver disease are emerging from laboratory studies of hereditary tyrosinemia type I—an inherited metabolic disorder associated with severe liver disease in infants and children. It is caused by a deficiency in an enzyme that breaks down the amino acid tyrosine, resulting in elevated tyrosine levels in the blood (tyrosinemia) and tissue damage. A drug for treating this disease was approved in 2002 which provides a means of long-term control of tyrosinemia. In cases of advanced disease, however, liver transplantation is the only effective current therapy. One way to correct the underlying defect in genetic diseases such as hereditary tyrosinemia would be through transplantation of cells with a functioning copy of the gene for the missing enzyme. In recent years, researchers have explored this possibility in mice that are deficient in the same enzyme that causes hereditary tyrosinemia type I in humans. They found that transplantation of stem cells derived from the bone marrow of healthy adult donor mice into the mice with tyrosinemia resulted in fusion between the healthy and diseased cells, correction of the genetic defect, and repair of the liver. However, the question remained of whether stem cells, with their ability to turn into a variety of cell types, were required, or if more mature cells—already committed to forming a particular cell type—could also work to correct the defect. Researchers addressed this by conducting a series of transplantation experiments using several different types of donor mice that were genetically engineered to produce only certain types of cells that originate in the bone marrow. They found



As a result of new insights into the understanding of the underlying causes of celiac disease, and in order to improve awareness, diagnosis, and management of this condition, in June 2004 the NIDDK and several other Institutes and Centers of the NIH convened a Consensus Development Conference on Celiac Disease. This conference examined the current state of knowledge and identified directions for future research. The 13-member panel included practitioners and researchers in gastroenterology, pediatrics, pathology, internal medicine, endocrinology, a dietitian, a geneticist, and a consumer representative. The panel made recommendations for future efforts to study and treat celiac disease. Illustration courtesy of the NIH Office of Medical Applications of Research, which co-sponsored the meeting and commissioned the image as part of the Conference.

that it was possible to correct the defect in mice with tyrosinemia by using macrophages—a kind of immune cell that develops from cells that form in the bone marrow. These results support the theory that donor stem cells used in prior experiments probably differentiated into macrophages prior to fusing with the recipient's liver cells. Importantly for potential clinical applications, this study also suggests that, in contrast to bone marrow transplantation, treatment with macrophages could be a less invasive, more efficient type of cell transplantation procedure for genetic liver diseases, such as hereditary tyrosinemia. A key benefit of macrophages or their immediate precursor cells is that they could be administered directly into the liver or bloodstream.

Willenbring H, Bailey AS, Foster M, Akkari Y, Dorrell C, Olson S, Finegold M, Fleming WH, and Grompe M. Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. *Nat Med* 10: 744-748, 2004.

While research aimed at determining ways to allow patients with end-stage liver disease to regenerate liver tissue holds hope for the future, researchers are seeking ways to expand the pool of organs available for transplant. Presently, more than 5,000 liver transplants are performed every year. Unfortunately, more than 17,000 patients are awaiting liver transplantation, and in recent years, the waiting list has continued to grow. As a consequence, the numbers of patients dying while on the liver transplant waiting list has grown.

Because of the shortage of donor livers available from cadavers, transplants from living donors have been the subject of much interest. While living donor liver transplantation has become widely accepted in pediatric patients, its use in adults is controversial, as the procedure is challenging and potentially dangerous. Between 1998 and 2003, at least two healthy, adult donors died after adult-to-adult living donor liver transplantation surgery.

To address the issues of the proper use, relative risks, and potential benefits of adult-to-adult living donor liver transplantation, the NIDDK established a multi-center clinical study. The “Adult-to-Adult Living Donor Liver Transplantation Cohort Study” (A2ALL) consists of nine liver transplant centers experienced in performing living donor liver transplantation and a data coordinating center responsible for maintaining the database on patients. The primary goal of A2ALL will be to provide valuable information on the outcomes of living donor liver transplantation. This important study will follow both donors and recipients before and after the liver transplant operation, assessing clinical outcomes and quality of life. This information will help aid decisions made by physicians, patients, and potential donors.

Combination Drug Therapy Effective for Hepatitis C – Research Advance from the NIDDK’s HALT-C Clinical Trial:

The hepatitis C virus (HCV) is the most common cause of liver disease in the US. About four million Americans have been infected with HCV, and most of them now have chronic hepatitis. In some, chronic hepatitis C leads to cirrhosis, liver failure, and liver cancer. Liver failure due to chronic hepatitis C is the most common cause of liver transplants. The only treatment proven to be effective for hepatitis C is interferon, with or without the antiviral drug ribavirin. Unfortunately, many patients treated with these medications do not respond, and the virus continues to cause liver damage. The NIDDK is supporting studies to determine if continuing interferon long-term in patients who remain infected with HCV may prevent progressive liver damage. The “Hepatitis C Antiviral Long-Term Treatment against Cirrhosis” (HALT-C) clinical trial is designed to determine if long-term treatment with pegylated interferon—a form of interferon chemically modified to make it longer-acting—in people with HCV who have not responded to previous interferon-based therapy can prevent cirrhosis and reduce the risk of developing end-stage liver disease and liver cancer. Patients enrolled in HALT-C initially receive a 24-week course of a combination of pegylated interferon and ribavirin, an antiviral drug. Patients who respond to this combination therapy continue for another 24 weeks; those who do not respond are randomized either to continue to receive pegylated interferon or to stop treatment. Patients will be followed for up to four years.

HALT-C researchers have recently reported the results of an early portion of the study. They asked whether patients with HCV who had previously been treated with—and not responded to—unmodified interferon alone would respond favorably to the combination therapy. Over 600 patients who were nonresponders to previous interferon therapy, with or without ribavirin, were treated with pegylated interferon plus ribavirin. After 20 weeks, 35 percent of the patients had no detectable evidence of HCV

infection in their blood. These patients continued on therapy for a total of 48 weeks. Following discontinuation of therapy, 18 percent of the initial number of patients achieved a sustained virologic response, which means that the virus remained undetectable in their blood. This study suggests that some patients who did not respond to initial therapy with interferon may benefit from re-treatment with pegylated interferon and ribavirin.

Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, Lok AS, Morgan TR, Bonkovsky HL, Lee WM, Dienstag JL, Ghany MG, Goodman ZD, and Everhart JE, and The HALT-C Trial Group. Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 126: 1015-1023, 2004.

The NIDDK is also pursuing multiple avenues of research into therapies for hepatitis C in diverse populations. It is known that African Americans with hepatitis C respond less well to interferon-based therapies, compared to Caucasians. In fact, several studies have found that the sustained response rate to interferon among African Americans is one-third to one-half of that seen in whites. To investigate possible reasons for this difference and identify possible improvements in treatment regimens, the NIDDK has funded the “Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C” (Virahep-C). This is a clinical trial designed to investigate the best available medication for treating hepatitis C, to see how well these medications work in African Americans and Caucasian Americans, and to study the reasons that treatment for hepatitis C works for some patients, but not for others. Patients are being treated with a combination of pegylated interferon and ribavirin for one year. Patients are then followed for another year after treatment. The Virahep-C trial may provide important information about how well African Americans respond to treatment for hepatitis C compared to Caucasian Americans; factors that may predict response to

treatment, especially in African American patients; and how the patients' genes, especially those controlling the immune system, affect response to treatment. The trial is expected to be completed in 2006.

The NIDDK is also concerned about the special problems of children infected with the hepatitis C virus. In the recently-launched clinical trial "Peginterferon and Ribavirin for Pediatric Patients with Chronic Hepatitis C" (Peds-C), approximately 120 children will be randomly assigned to receive either pegylated interferon alone or pegylated interferon and ribavirin for 48 weeks. The children are carefully monitored for evidence of liver disease and hepatitis C virus levels, as well as for any side effects of therapy, their growth and development, and quality of life. A long-term follow up study of the clinical trial participants is planned. This study is also receiving support from the Food and Drug Administration, as well as from industry.

Drug-induced Liver Injury: Every year, many people inadvertently suffer severe liver injury from prescription and "over-the-counter" medications, nutritional supplements, alternative medicines, and herbal preparations. Most drugs are safe for the majority of patients taking them, and the reason that some patients are susceptible to liver injury from a drug is rarely known. Drug-induced liver injury occurs in all age groups, but most cases are seen in older people, because they take more medications than younger persons and also use multiple medications. Their ability to metabolize drugs in the liver may also be less than that of younger people. Unfortunately, the extent and magnitude of the problem are not well understood, because definitions and data for drug-induced liver injury are suboptimal. To address this gap in knowledge, the NIDDK has launched a "Drug-induced Liver Injury Network" (DILIN). One objective of the Network is to develop standardized definitions and tools to identify and fully characterize cases of drug-induced liver injury. With systematic classification, researchers will be better able to analyze drug-induced liver injury and collect biological samples from patients that can then be used to

study the causes of liver toxicity. Another objective of the Network is to establish a registry of patients who have experienced severe drug-induced liver injury. The Network should enable researchers to develop better ways to prevent, detect, and treat this growing liver problem.

CELIAC DISEASE

Children at Risk for Celiac Disease May Have Subclinical Symptoms: People with celiac disease develop severe digestive problems when they eat gluten, a major protein component of grains such as wheat, rye and barley. A small amount of any of these foods is all that is required to damage the intestines of susceptible individuals, limiting the absorption of vital nutrients. Malabsorption slows physical development in children, and can cause a host of other symptoms. Definitive testing for celiac disease requires an intestinal biopsy, and the disease often goes undiagnosed. However, a blood test can identify at-risk individuals. Thorough screening of children born in Denver, Colorado, between 1993 and 1999 has shown that about 0.9 percent of children develop the disease by age five. A new study compares 18 children found to be at-risk, but who had not developed overt celiac disease, to 100 age- and gender-matched controls. The at-risk children had higher rates of some celiac disease symptoms, including irritability/lethargy and abdominal distension/gas, and were found to grow more slowly than their peers. These differences were small but statistically significant.

Hoffenberg EJ, Emery LM, Barriga KJ, Bao F, Taylor J, Eisenbarth GS, Haas JE, Sokol RJ, Taki I, Norris JM, and Rewers M. Clinical features of children with screening-identified evidence of celiac disease. *Pediatrics* 113: 1254-1259, 2004.

Hoffenberg EJ, MacKenzie T, Barriga KJ, Eisenbarth GS, Bao F, Haas JE, Erlich H, Bugawan TL, Sokol RJ, Taki I, Norris JM, and Rewers M. A prospective study of the incidence of childhood celiac disease. *J Pediatr* 143: 308-314, 2003.

Consensus Development Conference: In June 2004 the NIH convened a Consensus Development Conference on celiac disease. The 13-member panel included practitioners and researchers in gastroenterology, pediatrics, pathology, internal medicine, endocrinology, a dietitian, a geneticist, and a consumer representative. The panel reviewed an extensive collection of medical literature related to celiac disease. The panel concluded that celiac disease is under-diagnosed, and recommended increasing physician awareness of its various manifestations and appropriate use of available testing strategies. These proposed changes may lead to earlier diagnosis and better outcomes for patients. Based on its assessment of an extensive collection of medical literature and expert presentations, the panel identified six elements essential to treating celiac disease once it is diagnosed: (1) consultation with a skilled dietitian, (2) education about the disease, (3) lifelong adherence to a gluten-free diet, (4) identification and treatment of nutritional deficiencies, (5) access to an advocacy group, and (6) continuous long-term follow-up.

To address the recommendations of the Consensus Conference, the NIDDK is planning in early 2005 to discuss the development of a celiac disease awareness program with stakeholders—including patient advocates. The objective of such a program would be to increase the likelihood that primary care providers would recognize and take appropriate diagnostic steps for patients who might have celiac disease. Such an effort would address the Consensus Conference's finding that celiac disease is largely undiagnosed in the U.S., possibly because initial recognition of the disease is most likely to occur in the primary care setting.

BACTERIA IN THE GUT

Although many strains of bacteria cause illness, not all bacteria are unwanted; “good” bacteria live throughout the body with benefit to both host and microbe. When one thinks of bacteria, disease-causing microbes may be the first to spring to mind, such as the ones responsible for urinary tract infections, for example. However, the environment is full of bacteria that are benign or beneficial. Indeed, it has been estimated that between 500 and 1,000 different species of bacteria inhabit the human digestive tract. While the relationship between the body and disease-causing bacteria is relatively well understood, less is known about how the body and its associated “good” bacteria influence each other. In some conditions, for unknown reasons, the body mounts an immune response against “good” bacteria, triggering an inappropriate reaction that may contribute to conditions such as Crohn’s disease. NIDDK-supported scientists are working to better understand the relationship between both good and bad bacteria, and the body’s immune system, and protection against or vulnerability to disease.

Symbiotic Bacteria May Promote Intestinal

Health: The remarkably complex microenvironment of the intestine contains an abundance of microorganisms that provide health benefits to the host by inducing tolerance to substances in the environment or food that trigger an immune response. Although the gut is the site of rapid turnover of cells and propulsion of food and water, some microbes are able to latch-on to and colonize the intestine. Scientists continue to search for factors that differentiate microbial “residents” (those that successfully colonize)

versus “tourists” (those that merely pass through the digestive system). The factors of interest are those that can promote initial gut attachment and resistance to the wash-out of beneficial bacteria, as well as factors that inhibit colonization of harmful ones. Recent studies suggest that the polysaccharide-rich mucus gel layer of the human intestinal wall provides a matrix capable of supporting a thin layer of helpful bacteria that functions to aid digestion of intestinal contents and augment host defenses against disease causing organisms. Events leading to the interruption of this symbiotic relationship may promote an immune response to specific microbes. Emerging data indicates that *B. thetaiotaomicron*, a predominant member of the intestinal bacteria, induces intestinal Paneth cells to secrete a protein called angiogenin-4. Angiogenin-4 kills certain types of bacteria and may function to prevent microorganisms from invading the intestinal lining. Hence, specific bacteria of the gut may regulate expression of natural antibiotics and regulate the microbial ecology of the intestine. These findings may lead to the development of therapeutic and preventative strategies to support beneficial bacteria or impede the effects of those that cause disease.

Sonnenburg JL, Angenent LT, and Gordon JI. Getting a grip on things: how do communities of bacterial symbionts become established in our intestine? *Nat Immunol* 5: 569-573, 2004.

A Gene Expressed in Paneth Cells May

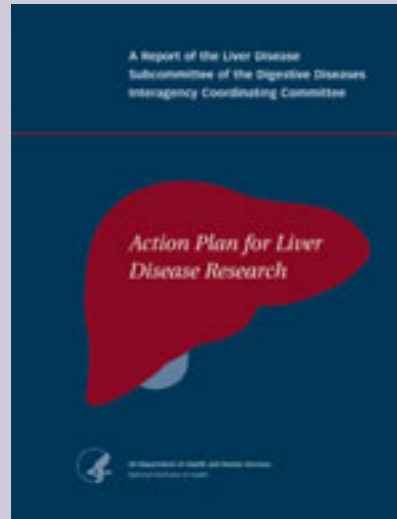
Contribute to Crohn’s Disease: Crohn’s disease is a chronic, currently incurable digestive disease, most commonly affecting either the colon or the portion of the small intestine nearest to it, the ileum. Symptoms frequently include abdominal pain, nausea, vomiting, weight loss, and diarrhea, which is occasionally bloody. The precise causes of Crohn’s disease are unknown, but bacteria in the gut are thought to contribute. There may also be a genetic component: the disease not only runs in families, but Americans and Europeans with the disease also frequently have particular variants of a gene called *card15*, which is expressed in immune cells, and believed to have a role in innate immunity to bacteria. In a new study, researchers found that the *card15* gene is expressed at high levels in so-called Paneth cells, which lie at the base of invaginations in the small intestine. The Paneth cells secrete anti-microbial compounds, probably playing an important role in controlling gut bacteria. Thus, *card15* and Paneth cells represent an apparent link between the genetic and bacterial risk factors for the illness, and are a promising target for development of therapeutics.

Ogura Y, Lala S, Xin W, Smith E, Dowds TA, Chen FF, Zimmermann E, Tretiakova M, Cho JH, Hart J, Greenson JK, Keshav S, and Nuñez G. Expression of NOD2 in Paneth cells: a possible link to Crohn’s ileitis. *Gut* 52: 1591-1597, 2003.

Advancing Liver Disease Research Across the NIH: The Action Plan for Liver Disease Research

Liver and biliary diseases affect Americans of all ages and walks of life. An estimated 5.5 million Americans currently have chronic liver disease or cirrhosis, while more than 20 million have gallbladder disease. Progress in controlling liver and biliary disease depends largely on advances in understanding of these diseases through biomedical research. An *Action Plan for Liver Disease Research* has been developed to respond to the need to advance research on liver and biliary diseases, with the ultimate aim of decreasing the burden of these diseases in the United States. The focus of the trans-NIH Action Plan is on identifying areas of scientific opportunity leading to practical but important goals in the prevention and control of liver and biliary diseases that could be pursued with NIH support over the next decade. The *Action Plan* addresses the broad range of liver disease research, and is organized around 16 topic areas:

- Cell and Molecular Biology of the Liver;
- Liver Injury, Inflammation, Repair, and Fibrosis;
- Developmental Biology and Regeneration;
- Bile, Bilirubin, and Cholestasis;
- Viral Hepatitis;
- HIV and Liver Disease;
- Fatty Liver Disease;
- Drug- and Toxicant-Induced Liver Disease;
- Autoimmune Liver Disease;
- Pediatric Liver Disease;
- Genetic Liver Disease;
- Liver Transplantation;
- Complications of Liver Disease;
- Liver Cancer;



The *Action Plan for Liver Disease Research* aims to advance NIH-supported research on liver diseases with the ultimate goal of decreasing their burden in the United States. It describes recent research advances and identifies important research goals to pursue over the next decade. The *Action Plan* was developed with broad external input from the research, professional, and patient-advocacy communities. Its development was directed by the Liver Disease Subcommittee of the statutory Digestive Diseases Interagency Coordinating Committee.

- Gallbladder and Biliary Disease; and
- Liver Imaging and Biotechnology.

The *Action Plan for Liver Disease Research* was developed in response to congressional interest in bringing additional focus to liver research supported by the NIH and in response to the public health burden of digestive diseases and emerging research opportunities to address them. The NIH made a commitment to building on the robust liver research portfolio in order to bring greater focus and coordination to liver disease research

supported by the NIH. A special focus on liver disease research was initiated by NIDDK in July 2003, when the Institute's Director established the Liver Disease Research Branch within the Division of Digestive Diseases and Nutrition (DDDN) of the NIDDK. The NIDDK Director appointed an internationally-recognized authority in liver disease to lead this Branch, which pursued the formation of a Liver Disease Subcommittee within the statutory Digestive Diseases Interagency Coordinating Committee (DDICC). This committee is a group of representatives from across the NIH and other Federal agencies that serves to coordinate research efforts combating digestive diseases.

The Action Plan represents the broad input of a diverse and talented group of individuals who are committed to advancing liver disease research, including those from the NIH and other Federal agencies, as well as intramural and extramural researchers, physicians, and representatives of professional and patient advocacy groups. This broad input was gained through several modes, including an open meeting, Working Groups and Primary Review Groups, and an invitation for public comments on the draft Action Plan through the Internet. The Action Plan can be accessed in electronic form through its website: <http://liverplan.niddk.nih.gov>. Hard copies of the publication are also available, and ordering information is

provided on the website. The plan also outlines ten research goals, or "benchmarks," to gauge overall progress in advancing liver disease research. The benchmarks are to:

- Improve the success rate of therapy of hepatitis C;
- Develop effective therapies that can be used in fatty liver disease, both alcoholic and nonalcoholic;
- Develop regimens of antiviral therapy that are effective in the long-term management of hepatitis B;
- Develop sensitive, specific, and noninvasive means of assessing disease stage (i.e., extent of fibrosis) in chronic liver disease;
- Develop sensitive and specific means of screening individuals at high risk for early hepatocellular carcinoma;
- Develop means to prevent gallstones;
- Elucidate the cause of biliary atresia;
- Improve the safety and define optimal use of living donor liver transplantation;
- Develop standardized and objective diagnostic criteria of major liver diseases and their grading and staging; and
- Decrease the mortality rate from liver disease.

Allen Russell

Liver Transplant for Alpha-1 Antitrypsin Deficiency Affords a New Lease on Life

Allen Russell's life was pretty much free of any serious illnesses. However, about the time he reached his mid-40s, he started experiencing shortness of breath. An allergist said it was related to asthma and started Allen on therapeutic inhalants. Allen, a smoker since his college days, saw a correlation and decided that it was time to quit. However, despite using the inhalants and quitting cigarettes, Allen's condition did not appear to improve.

In the spring of 1999, in follow-up to an earlier, unrelated blood test that had indicated slightly abnormal liver enzyme levels, Allen had a liver biopsy. Test results from the biopsy came back positive for alpha-1 antitrypsin deficiency (AAT deficiency, also called Alpha-1), one of the most prevalent, potentially lethal hereditary disorders. AAT deficiency can cause life-threatening lung disease and/or liver disease in adults and children.

"My gastroenterologist said that, in his more than 20 years of practice, he had never seen a case of alpha-1 antitrypsin deficiency before this," says Allen. But the physician did encourage Allen to see a hepatologist. This specialist later informed Allen that, given the results of his biopsy and several other tests, he would most likely need a liver transplant in 3 to 10 years. The diagnosis took Allen entirely by surprise. A former athlete, he always felt that he was the picture of health. In September 2002, just 3 years and 4 months after his diagnosis, Allen's life was saved by a liver transplant.

Today, Allen is doing well with his new liver and leading a very active life, much of it devoted to promoting organ donations and helping others get through



Photo credit – Rick Brady

Allen Russell

serious health problems. He is very grateful to the anonymous donor whose liver he received and to the donor's family. "Up until my transplant I thought my faith was strong," says Allen. "The transplant only bolstered my faith and my need to help others." But it was a long and scary journey for Allen and his family before getting to that point.

About Alpha-1 Antitrypsin Deficiency

Alpha-1-antitrypsin, or AAT, is a protein produced mostly by the liver. Its primary function is to protect the lungs from an enzyme, neutrophil elastase, that normally digests damaged or aging cells and bacteria in order to keep the body healthy. If there is insufficient AAT circulating in the bloodstream, the destructive action of the damaging enzyme is left largely unchecked, leading to the destruction of healthy lung tissue and resulting in conditions such as emphysema, chronic bronchitis and lung infections.

However, while too little AAT is responsible for lung damage in this disease, “too much” AAT may be responsible for damage to the liver. In the most common form of symptomatic AAT deficiency, an alteration in the AAT protein that inhibits its secretion from cells in the liver—and hence, reduces AAT levels in the bloodstream—also leads to its abnormal accumulation within liver cells. It appears that this buildup of AAT induces cellular responses that can eventually injure the liver cells and cause overall liver damage, such as cirrhosis. Because liver damage only occurs in a subset of AAT deficient patients, it is thought that other factors, environmental and/or genetic, also influence how the liver cells handle the buildup of AAT and thereby play a role in determining whether a patient progresses to liver disease. Thus, the same inherent flaw in the AAT protein may inflict damage on two major organs—the lungs and liver—by entirely different but intimately linked mechanisms. (See also “Story of Discovery: Flaws in Protein Processing: Insights from Alpha-1 Antitrypsin Deficiency.”)

Although it can be diagnosed in adulthood—Allen was diagnosed at age 46—AAT deficiency is an inherited disease and the most common genetic cause of liver disease in children. It most often appears in the newborn period with jaundice, swelling of the abdomen, and rejection of food.

According to the Alpha-1 Foundation, a not-for-profit organization dedicated to finding a cure for AAT deficiency, it is estimated that 20 million Americans are undetected carriers of the disease-causing gene and may be at risk for lung or liver disease; 100,000 individuals are actively lung- or liver-affected with fewer than ten percent (10,000) accurately diagnosed. Fortunately, for reasons still not understood, only 10 to 20 percent of those diagnosed with the deficiency will progress to liver disease. Although certain abnormalities associated with the liver disease can be treated or controlled, primarily with vitamin supplements, there is no cure for AAT deficiency.

For those patients who do suffer progressive liver damage to end-stage liver disease, the only option for survival, as Allen learned, is a liver transplant.

Living with AAT Deficiency

Allen was born with a severe case of jaundice and had to be re-hospitalized for 3 to 4 days only 6 weeks after his birth. “I didn’t have much of an appetite, and what I did eat, I’d throw up instantly,” he says. “The doctors didn’t know if my bile ducts were functioning or not. The ducts eventually began to function normally.” The acute problems soon passed, and because there is no known history of AAT deficiency in Allen’s family, the incident was never linked to the disease. In fact, AAT deficiency was not even named until the late 1960s, about 14 years later.

For decades, Allen never gave a second thought to his neonatal bout with his jaundice and proceeded to lead an active, productive life. In addition to swimming, playing baseball, golf and tennis from boyhood to high school, Allen says he played scout team quarterback for the Georgia Tech Freshman Football Team, and was the second fastest miler on that squad. “I had no clue anything was wrong with either my lungs or my liver,” he says. After college, Allen married, had two sons, and started out on a successful professional career in management with South Central Bell in Birmingham, Alabama.

However, in 1996, at age 43, Allen was told that his liver enzymes were a bit out of the normal range and that this condition should be monitored, but it didn’t seem to Allen at the time to be very serious. In 1998, he began feeling shortness of breath and started on inhalants. Unbeknownst to Allen, AAT deficiency was affecting both his liver and lungs. A year later, he had the liver biopsy and was told he would need a liver transplant in 3 to 10 years. “I was stunned,” says Allen. At first, he only told his wife and parents. A few weeks later he told his boss and later broke the news to his two teenage sons that he would eventually need a new liver.

PATIENT PROFILE

Coping with the Diagnosis

Since 10th grade, Allen had kept a journal. With this new knowledge of his AAT deficiency and the looming transplant, he began writing in it more frequently and earnestly. Eventually, he created a network of very close and trusted friends and relatives to whom he often turned “to talk through some new twist” in his journey, or “to share good or bad news.” He also began researching AAT deficiency and liver transplants on the Internet, looking for whatever information he could find. “I even visited medical libraries,” he says. “Learning as much as I could is how I dealt with my fear.”

Allen’s disease continued to progress. A second liver biopsy indicated that his cirrhosis was worsening. Three months later a blood test indicated that he had a severe shortage of platelets, the part of the blood necessary for clotting. By early 2002, Allen was experiencing bleeding in his esophagus and pains in his lower abdomen. By mid 2002, as a side effect of his declining liver, he began losing focus at work. By this time, he already was on the waiting list to receive a liver transplant. With accelerating emphysema and an aneurysm discovered near his liver, he moved up the waiting list.

Life-Saving Transplant

By nature, Allen is an organizer and planner. Just in case a liver from a deceased donor did not become available in time, Allen had three viable and willing living donors lined up, including his brother, a cousin-in-law and a very good friend. One of these candidates could have contributed a part of his or her liver to Allen, in a procedure called living-donor liver transplantation. But on September 5, 2002, at precisely 9:08 a.m., as Allen vividly recalls, he answered the phone to learn that a liver was available for him. His relief resulted in tears, he says, and within about 6 hours he arrived at the Vanderbilt University Medical Center, his carefully selected location for the transplant. He received his new liver early

the next morning, within only a few blocks of his jaundiced birth about 49 years earlier.

“Dear Donor Family Member(s),

My name is Allen and I’m very fortunate to have received a liver from your family member.... The donation...has helped me survive and gives me hope for many more years of happiness with my family and friends. I am sincerely grateful for this gift, and truly appreciate any part you had in making this possible.”

Allen’s recovery from surgery was so remarkably fast and went so well that he was released from the hospital in a week, then from the nearby hotel tailored for patient rehabilitation just two weeks later. Having expected to remain in Nashville for at least 6 weeks, Allen was delighted! “That gave me a great bit of confidence,” he says. By the end of October, however, his body began rejecting the new liver. Three days on heavy doses of steroids managed to stop the rejection. However, Allen experienced another rejection episode in November. This time, he was placed on different anti-rejection drugs and, according to Allen, “they seem to have done the trick. I’ve had no rejection of my liver since. My body just had to get compatible with the right drugs. I remain extremely grateful to my donor for my second chance in life.” Although Allen’s new liver is functioning well and has provided the needed AAT protein to his lungs so that further lung damage can be diminished, transplantation is not the ultimate cure for all AAT deficiency patients. Moreover, a number of factors, such as the limited supply of cadaveric livers available for transplantation, a lengthy waiting list, and cost, can ultimately prevent patients in need of a transplant from receiving this gift. Thus, developing better treatments for AAT deficiency will depend upon continued research on the underlying disease mechanisms, as well as continued careful assessment of new approaches to liver replacement, such as living donor liver transplantation.

Says Miriam O'Day, the Alpha-1 Foundation's senior director of public policy, "The organizations that advocate for individuals with Alpha-1 have tried to raise awareness that this is a liver disease that usually manifests clinically as a lung disease, making collaboration across disciplines essential." She adds that the public investment in research being conducted at NIDDK into liver disease, and AAT deficiency specifically, remains critical. In addition, the Foundation has invested significant funds from private donors into solving this problem.

By December 5, 2002, just three months after his transplant operation, Allen returned to his employer of over 20 years, BellSouth, to work half days, and a week later, returned to a full workload. "I was so excited to be back that my boss put me in charge of our group's morale!"

Today, Allen lives life at full tilt. He is a board member for the Alpha-1 Association, a member-based nonprofit organization founded in 1991 to identify those affected by AAT deficiency and to improve the quality of their lives through support, education, and advocacy. (The Alpha-1 Association is distinct from the Alpha-1 Foundation.)

The Association served to launch Allen's quest for knowledge about the disorder, and got him involved in a research project that helped him understand his illness and ways to cope. He has been a volunteer speaker and health fair resource for Georgia LifeLink, which promotes organ donations. He writes articles about his transplant for various publications, including one for the American Liver Foundation, and he maintains a website describing his transplant experience.

Allen serves on the board of the Kiwanis Club of Peachtree City, Georgia, and is a deacon for the First Presbyterian Church of Peachtree City. He is an active Georgia Tech alumnus. Allen's most recent project is "The Lighthouse Team," an on-line support group for patients, friends and families seeking advice and encouragement to help get them through the many stages of liver disease, transplantation, and on the road to wellness.

"My life's mission is to help people feel that there really is a light at the end of *their* tunnel," says Allen, who, now nearly 52 years old, is grateful to be alive as a result of someone else's generosity and selflessness.

STORY OF DISCOVERY

Flaws in Protein Processing: Insights from Alpha-1 Antitrypsin Deficiency

Inherited deficiencies in just a single protein can be devastating to the liver and the lungs. This protein is alpha-1 antitrypsin, or AAT. Secreted into the bloodstream primarily by the liver, this protein helps the body by inhibiting the activity of a group of enzymes, which have the power to destroy tissues.

In the genetic disease known as alpha-1 antitrypsin deficiency, this very important protein is impeded from doing its job. In severe cases, the AAT protein does not complete its journey from the interior to the exterior of the liver cell for secretion into the bloodstream. Rather, it forms polymers—orderly chains of AAT units—which aggregate in a place within liver cells that acts as a check-point to ensure the quality-control of proteins. The retention of polymerized AAT within the cells can then wreak damage on the liver. On the other hand, if inadequate levels of AAT reach the bloodstream, the protein cannot perform its important protective role of keeping a critical enzyme in check. If uncontrolled, that enzyme can cause lung tissue destruction that often progresses to emphysema. Thus, damage can come from either too much or too little AAT activity in key tissues.

NIH-funded research has helped to decipher the clinical manifestations and genetic underpinnings of AAT deficiency. Although the disease is caused by a single abnormal gene, its manifestations vary greatly depending upon whether a person inherits copies of the abnormal gene from one or both parents, and also on unknown genetic modifiers that affect gene expression. About 100,000 Americans have the severe form of AAT deficiency,¹ which is the most common genetic cause of liver disease in children, and also predisposes adults to chronic liver disease and liver

cancer.² Over 100 variants of the AAT gene have been discovered and grouped into three categories based on the level of AAT in the bloodstream—normal, deficient, or virtually undetectable.

With knowledge gained from research has come an expanded understanding of AAT deficiency disease, the differences among its various forms, and development of therapeutics based on this scientific foundation. The importance of AAT was originally recognized in studies of blood proteins when, in 1963, scientists found that the blood of emphysema patients lacked sufficient amounts of AAT. In 1966, other researchers observed that patients with a particular variant of the AAT gene have a high frequency of liver disease, including neonatal jaundice and cirrhosis. In addition, the patients had high concentrations of AAT in their liver cells. These and other observations about the disease enabled fundamental research to begin uncovering underlying mechanisms—a prelude to therapeutic development.

For example, in the 1980s, an important step forward in combating AAT lung disease was the demonstration that augmenting a patient's natural levels of AAT with externally administered protein was feasible and beneficial. AAT-deficient patients achieved an increase in their blood levels of the protein following the intravenous transfusion of purified AAT from the blood of healthy individuals. This finding led to FDA approval of the augmentation drug, Prolastin, which has become the most widely used treatment for AAT lung disease. Other related therapies on the horizon are intravenous augmentation products, inhalation delivery systems, and synthetic augmentation therapies.

Scientists supported by the NIH have focused intense research on liver damage arising from AAT deficiency disease. Researchers have searched for factors that might predispose patients to be susceptible to or protected from this liver damage. To this end, in 1994, researchers grew skin cells from AAT-deficient individuals who had never suffered from liver disease and who therefore might be “protected.” Similar cultures were made with cells from AAT-deficient individuals who had severe liver disease and were therefore considered “susceptible.” While cells from both cultures accumulated the AAT protein, only the “susceptible” cells exhibited a delay in degrading AAT, suggesting that some AAT-deficient patients have alterations in the degradation pathway for the protein in their liver cells—alterations that may predispose them to developing liver disease.

Additional research supported by the NIH substantiated, in 1989, that the aggregation of AAT protein within liver cells causes liver disease. The foundation for this discovery was a study of genetically engineered mice that produced sufficient AAT to protect them from lung disease, yet the mice still suffered from a build up of AAT in their liver cells. This finding led to research aimed at improving secretion of AAT, and pointed to discrete steps in protein processing that might be used as therapeutic targets. Researchers hope to build on previous NIH supported studies in which compounds known as “chemical chaperones” have been shown to be capable of inducing AAT secretion into the bloodstream. In related research, studies have shown that patients with AAT deficiency sustain liver damage due to inflammatory immunological responses to the aggregated protein. The immunosuppressive drug cyclosporin A was shown to prevent AAT liver damage—a proof-of-principle for mechanism-based therapeutic approaches to AAT deficiency. These types of studies may lead to the testing of various drug combinations to arrest or mitigate one or more of the sequential steps in the cascade of events that culminates in liver cell injury.

A new research impetus comes from the study of small molecules that can be used therapeutically to ameliorate protein processing defects, such as those seen in AAT deficiency disease. It is expected that diseases involving abnormalities in protein processing—including AAT, cystic fibrosis, and others within the NIDDK mission—will benefit from the NIH Roadmap Initiative to develop libraries of small molecules with therapeutic potential. Knowledge about AAT deficiency and similar diseases is also being advanced by more general research conducted during the 1970s and 1980s on protein degradation. Protein processing and the degradation of old and malformed proteins are now acknowledged as essential in maintaining healthy cells. The Nobel Prize in Chemistry was awarded in 2004 in recognition of these discoveries. The NIH is proud that its sustained support of this research led to the prize-winning findings. Because AAT deficiency is an inherited disease, researchers also continue to pursue potential therapeutic approaches that could correct the genetic abnormality responsible for deficiencies in the protein. These approaches include laboratory studies of gene therapy and gene repair, as well as stem-cell approaches. The inclusion of AAT deficiency in the recently funded Cholestatic Liver Disease Consortium, jointly funded by the NIDDK and the Office of Rare Diseases, provides an opportunity to gather clinical and biochemical data and an adequate number of biosamples in a prospective manner to stimulate research on the pathogenesis and optimal diagnosis, as well as chemoprevention and treatment of this disease. The rich variety of therapeutic approaches to AAT deficiency reflects both the complexity of the disease and the expanding possibilities for multiple types of interventions to arrest or ameliorate its underlying processes.

¹ Sandhaus RA. Alpha1-antitrypsin deficiency - 6: New and emerging treatments for alpha1-antitrypsin deficiency. *Thorax* 59: 904-909, 2004.

² Perlmutter DH. Liver injury in alpha1-antitrypsin deficiency: an aggregated protein induces mitochondrial injury. *J Clin Invest* 110: 1579-1583, 2002.